GENERAL INSTRUCTIONS FOR SUBCUTANEOUSLY IMPLANTED XENOGRAFTS (J2 PROTOCOL)

Revised August 9, 1993

ANIMALS:

Propagation: Athymic random bred (NCr-nu) mice.

Testing: Athymic random bred (NCr-nu) mice.

Weight: Mice should have a minimum weight of 18 g for males and 17 g for females.

Sex: One sex is used for all animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted in comments.

EXPERIMENT SIZE:

General Testing:
6-10 animals per test group.

Control Group: A minimum of 20 control animals should be used.

TUMOR TRANSFER FOR PROPAGATION AND TESTING:

Fragment: Prepare a 30-mg (acceptable range 20-40 mg) fragment from 300-1000 mg sc donor tumor without ulceration.

Suspension: Prepare a suspension of diluted ascitic fluid so that a 0.1ml portion contains 1 X 10^6 cells.

Site: Implant sc either 0.1 of suspension containing 1 X 10^6 cells or a 30-mg fragment into axillary region with puncture in inguinal region. Implant sufficient animals so that tumors may be selected in the proper size range.

Tumor Stage: When the median tumor size reaches 200 mg (the range of individual tumor sizes should be 100-400 mg) or as specified.

TUMOR STAGING AND TEST REQUIREMENTS:TEST REOUIREMENTS:

Staging Day: Measure tumors to the nearest 1.0 mm. Record tumor measurements (mm) and animal weights (g) for individual mice on staging day (SD). Randomize the selected mice (bearing tumors in the specified range) and individually identify mice as appropriate.

Deaths: Record deaths daily.

Animals Weights and Tumor Measurements: Weight animals and measure tumors twice weekly.

Treatments: Administer test agent based on the individual body weight on the specified day of treatment. See attached table for general treatment schedules.

Toxicity: Record individual animal weights on the tumor measurement days. If mice have a 20 % or greater loss in body weight not associated with tumor growth, the dose should be considered toxic.

Early Sacrifice: If individual tumors approach 5 g or more, the mice should be sacrificed and tumor dimensions and animal weight recorded.

Evaluation Day: At least 14 days after the last treatment, unless tumor growth requires earlier sacrifice, end and evaluate the experiment. Record individual tumor measurements and animal weights.

Evaluation of Activity:

The following parameters will be recorded/calculated:

- number of tumor-free animals
- number of drug-related deaths
- number of no takes
- number of partial regressions
- number of complete regressions
- optimal delta T/delta C%
- % T-C/C
- median days to achieve a defined tumor weight or number of tumor doublings
- net log cell kill

In Vivo Characterization and Reference Data for SC Human Tumor Xenographs (May 14, 1993)

Tumor Code	Tumor Line	Time to Reach 100-400mg[1] (days)	DT at 200-400mg days (Prior 2 Tests)	DT at 200-400mg days (Last Test)	Current Schedule for drug Evaluation [2,3] Treatments	Current Schedule for drug Evaluation [2,3] Measurements per week	Suggested Schedule Treatments	Suggested Schedule Measurements per Week
QH	UCSD 535L	15-20	(7.4, 7.3)	3.0	q4dx3 (SD)	2	No change	
*JP	DLD-1	10-14	(3.9, 4.5)	3.1	q4dx3 (SD)	2	No change	
JA	NCI-H23	10-20	(4.5, 4.0)	3.3	q4dx3 (SD)	2	No change	
	MLI-045	17-22	(3.3, single)	3.3	q4dx3 (SD)	2	No change	
	BZRT-33	10-14	(5.2, 5.0)	3.3	q4dx3 (SD)	2	No change	
*YV	KM12	8-12	(2.8, 2.8)	3.3	q4dx3 (SD)	2	No change	
*BG	SNB-75	16-20	(3.7, 2.9)	3.5	q4dx3 (SD)	2	No change	
*JR	DMS-114	15-20	(3.6, 4.6)	3.7	q4dx3 (SD)	2	No change	
*YL	SN12C	15-20	(10.7, 11.8)	3.7	q4dx3 (SD)	2	No change	
	UABCO2	17-20	(5.2, 5.0)	3.7	q4dx3 (SD)	2	No change	
JK	NCI-H82	12-16	(4.2, 4.6)	3.7[4]	q4dx3 (SD)	2	No change	
*YG	COLO-205	20-27	(4.6, 2.3)	3.8	q4dx3 (SD)	2	No change	
JZ	SK-MES-1	12-16	(3.1, 2.8)	3.9	q4dx3 (SD)	2	No change	
*JX	Ovcar 5	14-18	(10.9, 9.3)	4.0	q4dx3 (SD)	2	No change	

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Tumor Code	Tumor Line	Time to Reach 100-400mg[1] (days)	DT at 200-400mg days (Prior 2 Tests)	DT at 200-400mg days (Last Test)	Current Schedule for drug Evaluation[2,3] Treatments	Current Schedule for drug Evaluation[2,3] Measurements per week	Suggested Schedule Treatments	Suggested Schedule Measurements per Week
*YP	SK-Mel-28	3-7	(2.4, 1.1)	1.3	q4dx3 (SD)	2	qd1x5 (SD)	2
	Hop 27	6-9	(1.1, 2.2)	1.3	qd1x4 (SD)	3	qd1x5 (SD)	2
*TB	DMS 273	7-12	(1.9, 1.7)	1.6	qd1x4 (SD)	3	qd1x5 (SD)	2
YW	A-2780	7-10	(2.0, 3.1)	1.7	qd1x4 (SD)	3	qd1x5 (SD)	2
*LO	LOX-IMVI	5-7	(1.9. 2.1)	1.8	qd1x4 (SD)	3	qd1x5 (SD)	2
*QK	SF-295	7-11	(1.5, 1.6)	1.9	qd1x4 (SD)	3	qd1x5 (SD)	2
JW	SN 12K1	8-14	(2.2, 2.5)	2.0	qd1x4 (SD)	2	qd1x5 (SD)	2
KH	PC-3 Prostate	8-12	(2.0, 2.1)	2.0	qd1x4 (SD)	3	qd1x5 (SD)	2
ZA	LN CAP Prostate	6-15	(1.4, 1.6)	2.0	qd1x4 (SD)	3	qd1x5 (SD)	2
*YK	HCT-116	10-14	(2.5, 3.3)	2.0	qd1x4 (SD)	3	qd1x5 (SD)	2
	U-87 MG	6-9	(single)	2.1	q4dx3 (SD)	2	qd1x5 (SD)	2
YN	MHM-8 Sarcoma	8-15	(3.1, 2.8)	2.1[4]	qd1x4 (SD)	3	qd1x5 (SD)	2
YQ	KM12L4a	7-10	(2.0, 1.8)	2.3	q4dx3 (SD)	2	qd1x5 (SD)	2
*YE	CAKI-1	15-25	(2.3, 3.1)	2.4	qd1x4 (SD)	2	qd1x5 (SD)	2
*JO	SW 620	7-11	(3.1, 1.9)	2.4	qd1x4 (SD)	3	qd1x5 (SD)	2

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*BD	Molt-4 Leukemia	14-24	(3.4, 3.9)	2.0	q4dx3 (SD)	2	no change	
YU	KM12YR	10-14	(2.5, 2.7)	2.3	q4dx3 (SD)	2	no change	
*YJ	A498	14-16	(3.2, 3.3)	2.4	q4dx3 (SD)	2	no change	
TE	TE671	10-15	(3.0, 3.7)	2.5	q4dx3 (SD)	2	no change	
*QN	UACC 62	10-15	(2.5, 2.6)	2.7	q4dx3 (SD)	2	no change	
	A427	14-18	(3.3, 2.4)	2.7	qd1x4 (SD)	3	q4dx3 (SD)	2
YR	SNB-7	7-11	(2.4, 3.1)	2.7	q4dx3 (SD)	2	no change	
*QL	XF 498	13-15	(4.9, 5.6)	2.6	qd1x4 (SD)	3	q4dx3 (SD)	2
	SW-608	6-12	(2.5, 2.4)	2.7	q4dx3 (SD)	2	no change	
	UISO-BCA-1	16-19	(4.0, 5.4)	2.8	q4dx3 (SD)	2	no change	
*LV	NCI-H322M	10-20	(4.9, 5.3)	3.0	q4dx3 (SD)	2	no change	
	LXFL625	16-18	(5.3 only)	3.0	q4dx3 (SD)	2	no change	

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Tumor Code	Tumor Line	Time to Reach 100-400mg[1] (days)	DT at 200-400mg days (Prior 2 Tests)	DT at 200-400mg days (Last Test)	Current Schedule for drug Evaluation [2,3] Treatments	Current Schedule for drug Evaluation [2,3] Measurements per week	Suggested Schedule Treatments	Suggested Schedule Measurements per Week
*CL	NCI-H460	8-14	(1.9, 2.7)	2.5	q4dx3 (SD)	2	qd1x5 (SD)	2
QD	LS 180	8-11	(2.3, 1.9)	2.6	qd1x4 (SD)	3	qd1x5 (SD)	2
JC	NCI-H522	15-20	(1.9, 2.7)	2.8	q4dx3 (SD)	2	qd1x5 (SD)	2
*RF	RXF 393	5-8	(2.6, 1.3)	2.7	qd1x4 (SD)	3	qd1x5 (SD)	2
*QE	HCT-15	9-13	(2.6, 3.2)	2.5	qd1x4 (SD)	3	qd1x5 (SD)	2

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YO	COLO-320DM	10-14	(5.6, 6.2)	4.1	q4dx3 (SD)	2	no change	
	SHP-77	18-24	(4.3, 3.6)	4.3	q4dx3 (SD)	2	no change	
*QS	EKVX	16-20	(7.1, 3.6)	4.4	q4dx3 (SD)	2	no change	
QG	UCSD 354L	8-12	(4.3, 2.5)	4.5	q4dx3 (SD)	2	no change	
*BE	CCRF-CEM	14-24	(4.3, 4.6)	4.5	q4dx3 (SD)	2	no change	
JH	NCI-H69	10-15	(4.2, 4.5)	4.5[4]	q4dx3 (SD)	2	no change	
JM	LOVO	8-12	(8.9, 7.2)	4.6	q4dx3 (SD)	2	no change	
*QI	HCC-2998	12-18	(2.5, 2.2)	4.7	q4dx3 (SD)	2	no change	
	G298L	12-15	(11.5, 5.0)	4.7	q4dx3 (SD)	2	no change	
*QQ	SK-Mel-2	20-30	(6.1, 5.1)	4.8	q4dx3 (SD)	2	no change	
JD	NCI-H125M	10-20	(5.0, 5.4)	5.0	q4dx3 (SD)	2	no change	
*QR	SK-OV-3	20-30	(4.1, 4.5)	5.0	q4dx3 (SD)	2	no change	
QF	UCSD 242L	12-18	(4.2, 4.1)	5.2	q4dx3 (SD)	2	no change	
	A704	13-28	(7.6, 6.6)	5.2	q4dx3 (SD)	2	no change	
JG	NCI-H520	20-27	(4.3, 3.8)	5.2[4]	q4dx3 (SD)	2	no change	

In Vivo Characterization and Reference Data for SC Human Tumor Xenographs (May 14, 1993)

Tumor Code	Tumor Line	Time to Reach 100-400mg[1] (days)	DT at 200-400mg days (Prior 2 Tests)	DT at 200-400mg days (Last Test)	Current Schedule for drug Evaluation [2,3] Treatments	Current Schedule for drug Evaluation [2,3] Measurements per week	Suggested Schedule Treatments	Suggested Schedule Measurements per Week
*YH	IGROV-1	20-24	(6.6, 5.6)	5.3	q4dx3 (SD)	2	no change	
*TA	KM20L2	14-20	(2.8, 3.1)	5.4	q4dx3 (SD)	2	no change	
	CALU-6	12-16	(3.3, 4.0)	5.8	q4dx3 (SD)	2	no change	
*LN	A549	15-20	(10.9, 9.4)	5.8	q4dx3 (SD)	2	no change	
	MAXF 401	16-27	(6.1, 7.2)	5.8	q4dx3 (SD)	2	no change	
YT	SN12L1	12-18	(9.6, 5.9)	5.9	q4dx3 (SD)	2	no change	
CC	COLO 746	17-40	(12.7, 5.2)	5.9	q4dx3 (SD)	2	no change	

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QC	SK-MEL-31	20-30	(12.0, 6.1)	5.2	q7dx3 (SD)	1	q7dx3 (SD)	1
	UCSD 462L	16-20	(6.1, 7.2)	6.0	q4dx3 (SD)	2	q7dx3 (SD)	1
*UG	U-251	9-15	(6.7, 3.9)	6.1	q4dx3 (SD)	2	q7dx3 (SD)	1
JV	DU-145 Prostate	15-25	(9.9, 6.3)	6.1[4]	q4dx3 (SD)	2	q7dx3 (SD)	1
QJ	H498	11-14	(6.5, 4.4)	6.7	q4dx3 (SD)	2	q7dx3 (SD)	1
YS	SN12A1	10-15	(18.7, 4.1)	6.8	q4dx3 (SD)	2	q7dx3 (SD)	1
*YM	SNB-19	14-18	(6.6, 9.4)	7.3	q4dx3 (SD)	2	q7dx3 (SD)	1
	UABOV1	20-30	(7.5, 6.6)	7.4	q7dx3 (SD)	1	no change	
*QA	M14	15-25	(4.5, 11.8)	7.7	q4dx3 (SD)	2	q7dx3 (SD)	1
*YF	MALME-3M	12-15	(16.9, 16.2)	8.2	q7dx3 (SD)	1	no change	
*BB	HOP 92	20-25	(6.9, 12.5)	8.4	q4dx3 (SD)	1	q7dx3 (SD)	1
*QP	UACC 257	17-24	(5.8, 5.0)	8.6	q7dx3 (SD)	2	q7dx3 (SD)	1
*JQ	SK-Mel-5	15-25	(10.2, 5.4)	8.6	q4dx3 (SD)	2	q7dx3 (SD)	1
QB	SN12S1	15-25	(14.0, 6.0)	9.3	q7dx3 (SD)	1	no change	

In Vivo Characterization and Reference Data for SC Human Tumor Xenographs (May 14, 1993)

Tumor Code	Tumor Line	Time to Reach 100-400mg[1] (days)	DT at 200-400mg days (Prior 2 Tests)	DT at 200-400mg days (Last Test)	Current Schedule for drug Evaluation [2,3] Treatments	Current Schedule for drug Evaluation [2,3] Measurements per week	Suggested Schedule Treatments	Suggested Schedule Measurements per Week
*C2	HT29	12-18	(8.6 only)	9.4	q4dx3 (SD)	2	q7dx3 (SD)	1
	COLO 741	13-17	(14.6 only)	9.5	q7dx3 (SD)	1	no change	

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	MAXF 583	35-40	(10.8, 7.3)	11.9	q7dx3 (SD)	1	q7dx4 (SD)	1
*JY	Ovcar 8	20-30	(12.2, 12.5)	12.2	q7dx4 (SD)	1	no change	
	MAMGI-101	30-40	(12.0, 12.4)	15.9	q7dx4 (SD)	1	no change	
*QM	M19-Mel	50	(13.1, 17.7)	16.9	q7dx4 (SD)	1	no change	

1. To accommodate the narrow weight range for staged tumor size, 50% - 75% excess mice may have to be implanted with tumor fragment

2. Unless otherwise specified, activity is calculated by the delta method.

3. Host body weights are recorded on staging day (Weigh Day 1) and on tumor measurement days during treatment and for two weeks after the end of treatment. Weigh weekly thereafter. Tumor measurements must be made on the day of animal weights because the computer program calculates animal weight.

4. Calculated from median tumor weights determined on staging and evaluation days or on multiple days as available. Data were not available to calculate DT from 200 to 400 mg for individual tumors.

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* Indicates panel tumor line

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Tumors shown in **BOLD** indicate that tumor growth characteristics have changed significantly and a new treatment schedule is suggested for future experiments.

SPECIFIC DETAILS FOR J2 PROTOCOLS

TUMOR	DETAILS
CLJ2	PROPAGATION: 30 mg fragment (range 20-40 mg) from a 200-500 mg s.c. donor tumor (approximately day 10-14). Implant fragment in the axillary region with puncture in the inguinal region. Implant 50-75% additional tumors so that a range in tumor size can be selected on Staging Day. Staging Day ca day 10-14. Terminate experiment on Staging Day + 20.
JAJ2	PROPAGATION: 30 mg fragment (range 20-40 mg) from a 200-500 mg s.c. donor tumor (approximately day 20-28). Implant fragment in the axillary region with puncture in the inguinal region. Implant 50-75% additional tumors so that a range in tumor size can be selected on Staging Day. Staging Day ca day 20-28. Terminate experiment on Staging Day + 16.
JCJ2	PROPAGATION: 30 mg fragment (range 20-40 mg) from a 200-500 mg s.c. donor tumor (approximately day 18-25). Implant fragment in the axillary region with puncture in the inguinal region. Implant 50-75% additional tumors so that a range in tumor size can be selected on Staging Day. Staging Day ca day 18-25. Terminate experiment on Staging Day + 12.
JHJ2	PROPAGATION: 30 mg fragment (range 20-40 mg) from a 200-500 mg s.c. donor tumor (approximately day 14-21). Implant fragment in the axillary region with puncture in the inguinal region. Implant 50-75% additional tumors so that a range in tumor size can be selected on Staging Day. Staging Day ca day 14-21. Terminate experiment on Staging Day + 20.
JMJ2	PROPAGATION: 30 mg fragment (range 20-40 mg) from a 200-500 mg s.c. donor tumor (approximately day 12-18). Implant fragment in the axillary region with puncture in the inguinal region. Implant 50-75% additional tumors so that a range in tumor size can be selected on Staging Day. Staging Day ca day 12-18. Terminate experiment on Staging Day + 12.
LNJ2	PROPAGATION: 30 mg fragment (range 20-40 mg) from a 200-500 mg s.c. donor tumor (approximately day 20-25). Implant fragment in the axillary region with puncture in the inguinal region. Implant 50-75% additional tumors so that a range in tumor size can be selected on Staging Day. Staging Day ca day 20-25. Terminate experiment on Staging Day + 20.
JPJ2	PROPAGATION: 30 mg fragment (range 20-40 mg) from a 200-500 mg s.c. donor tumor (approximately day 9-13). Implant fragment in the axillary region with puncture in the inguinal region. Implant 50-75% additional tumors so that a range in tumor size can be selected on Staging Day. Staging Day ca day 9-13. Terminate experiment on Staging Day + 12.
JQJ2	PROPAGATION: 30 mg fragment (range 20-40 mg) from a 200-500 mg s.c. donor tumor (approximately day 20-40). Implant fragment in the axillary region with puncture in the inguinal region. Implant 100% or more additional tumors so that a range in tumor size can be selected on Staging Day. Staging Day ca day 20-40. Terminate experiment on Staging Day + 40.
JZJ2	PROPAGATION: 30 mg fragment (range 20-40 mg) from a 200-500 mg s.c. donor tumor (approximaely day 14-19). Implant fragment in the axillary region with puncture in the inguinal region. Implant 100% additional tumors so that a range in tumor size can be selected on Staging Day. Staging Day ca day 14-19. Terminate experiment on Staging Day + 12.
YOJ2	PROPAGATION: 30 mg fragment (range 20-40 mg) from a 200-500 mg s.c. donor tumor (approximately day 12-20). Implant fragment in the axillary region with puncture in the inguinal region. Implant 75-100% additional tumors so that a range in tumor size can be selected on Staging Day. Staging Day ca day 12-20. Terminate experiment on Staging Day + 12.